STUDIES OF THE CORN AMYLOSES.

III. The Isolation of Corn Gamma Amylose and Some Properties of Corn Beta Amylose Solutions.

DISSERTATION

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in
the Faculty of Pure Science of
Columbia University.

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To Mr. Ormond G. Smith my thanks are due for his generous gift.

W. L. E.

SEPARATION OF THE CORN AMYLOSES

INTRODUCTION

In the work carried on in this laboratory on the separation of the amyloses in purified corn starch it was noticed that in no case was all of the combined fatty acid accounted for in the amount of fatty acid bearing corn alpha amylose recovered. In this discussion corn alpha amylose is considered to be the fatty acid ester type of a complex carbohydrate which, for purposes of reference, I shall call corn gamma amylose, differing from the beta amylose (pure carbohydrate) in solubility and in other respects, as will be shown later. The following figures of the amount of combined fatty acid present, the combined fatty acid recovered in the form of alpha corn amylose and the calculated amount of fatty acid lost in the determination are taken from the results of previous work[1]. These quantitative figures form the basis for the statement made above.

TABLE I

Showing the loss of fatty acid in starch during treatment.

Milligrams of fatty acid in 10 grams of:—

	(A) Raw Starch	(B) HCl Treated Starch	(C) NH_4SCN Treated Starch	(D) Alpha Amylose	(E) Starch After Separation	(F) Starch Lost in Treatment	(G) Difference B - C	(H) Per Ct. of Fatty Acid Lost
Old Starch	65	47	30	160	24	41	17	65
Old Starch	67	47	30	150	23	44	17	66
New Starch	59	55	36	230	35	24	19	41
New Starch	56	54	35	230	35	21	19	38

Taylor and Nelson[2] show a decrease in fatty acid content when starch is treated with ammonium thiocyanate from 0.56% to 0.41%. In other words, 26% of the combined fatty acid is lost in the treatment.

Attention is directed to the uniform decrease in the fatty acid content after the whole starch has been subjected to the treatment with ammonium thiocyanate[1] whereas the difference due to the treatment with alcoholic hydrogen chloride is not uniform[1, 2]—see column 7.

The question naturally arises as to what the fate is of the carbohydrate part (gamma) of the alpha corn amylose lost in these separations.

The loss just mentioned affects to some extent the quantitative aspects of the separation[1]. Of greater interest, however, is the comparison of the properties of this carbohydrate with those of the alpha and beta amyloses.

If the carbohydrate part of corn alpha amylose is a new carbohydrate, gamma, then the methods of methylation and acetylation as applied by Werntz[3] on corn alpha amylose probably represent the formation of derivatives of this new amylose. Work on this phase of the subject is now in progress in this laboratory.

As a result of the present work, it is concluded that corn gamma amylose differs from alpha amylose in the following manner: it contains little or no fat; it is reversibly soluble in hot water; after treatment with aqueous alkali and subsequent neutralization it gives with iodine test solution the blue color characteristic of beta amylose. It differs from beta amylose as follows: it is insoluble in cold water; it is slowly soluble in cold concentrated hydrochloric acid; it does not give the blue iodine test when isolated. Further it is soluble in a molar solution of potassium iodide or ammonium thiocyanate to the extent of about 1 gram in 500 cc. and due to this property it is included in the corn beta amylose in the separation of the corn amyloses[1] where ammonium thiocyanate is used as the gelatinizing agent.

When the re-solution of fat free corn beta amylose is attempted there is always a portion which does not dissolve. This difficulty was first ascribed to the much discussed phenomenon of retrogradation or reversion of starch of which beta amylose forms the major portion. Apparently, however, a third form of amylose was present admixed with the beta amylose which has its genesis in alpha amylose by cleavage rather than in the beta amylose through chemical change.

In the process of separation of alpha and beta amyloses[1] there is a high concentration of ammonium thiocyanate present in the clear solution containing the beta amylose. When this beta amylose is precipitated it shows no fatty acid by hydrolysis and thus the possible presence of alpha amylose is disposed of. When the attempt is made to redissolve this corn beta amylose, after being washed free of ammonium thiocyanate, there is found a cloudy suspension which later precipitates and withstands all mechanical efforts to dissolve it. Since there is no further precipitation after this cloudy material settles out the inference is that there is an insoluble material present in the original corn beta amylose which had not been included in the alpha

amylose separated from it. As has been pointed out the only change in the condition of the two solutions before and after precipitation is the lesser amount of ammonium thiocyanate.

This cloudiness indicates the probable presence of insoluble gamma amylose previously defined as the complex carbohydrate part of corn alpha amylose. The following experiments will show the conditions just described.

EXPERIMENTS ON RE-SOLUTION

In order to determine the solubility of the crude corn beta amylose prepared by Taylor and Iddles (loc. cit.), solutions of thiocyanate free corn beta amylose were made up by adding the dry solid slowly to mechanically stirred water and bringing the suspension to about 90° C. at which point it became quite clear. It was always noticed that when the temperature reached about 87° C. there was a rather sudden and very distinct change in the opacity of the solution as noticed also by Sherman and Baker[4], and for this reason the mixture was always brought at least to this temperature. The solutions were cooled by standing at room temperature and allowed to settle over night. The clear supernatant liquid was ultrafiltered by a modification of the method of Taylor and Iddles and dry weight taken according to the procedure outlined below.

The following table shows the results of these experiments:

TABLE II

The Solubility of Crude Beta Amylose at 90° C.

Sample	Gms.	In cc. Water	Calc. Conc. mg. per cc.	Actual Conc. mg. per cc.	Per Cent Beta Amylose Dissolved
M	18	300	49.2	47.2	96
N	18	300	49.2	47.0	95
X	30	750	35.4	33.4	94
Y	20	500	35.4	33.2	94
Z	20	500	35.4	29.8	81

As a matter of interest, the comparative solubility of crude corn beta amylose at room temperature and at 90° C. is shown by the results in the following table. The beta amylose was stirred with the cold water for 2 and 6 hours respectively, allowed to settle, ultra filtered and a dry weight determination made on the clear filtrate. After the sample had been taken in the cold, the suspension mixture was heated to 90° C. at which point it became opalescent, allowed to settle, ultrafiltered and a dry weight determination made on the filtrate.

TABLE III

Showing the Comparative Solubility of Crude Beta Amylose.

Sample	Grams	In cc. Water	Calc. Conc. mg. per cc.	Actual Conc. mg. per cc.	Per Cent Beta Amylose Dissolved
N′	18 (cold)	300	49.2	33.4	68
N	18 (heated)	300	49.2	47.0	95
P′	13.5 (cold)	300	37.0	24.8	67
P	13.5 (heated)	300	37.0	35.0	93

In the dry weight determinations mentioned above the procedure was to take 10 cc. samples of liquid in previously weighed and dried dishes of clean ignited Ottawa sand. They were put in a Friess Oven at 90° C. for 2½ hours. The temperature was then raised to 110° C. and kept for 1 hour. The samples were then cooled in a dissicator over phosphoric anhydride for 1 hour and weighed. This procedure was followed in every case.

Trials made during the weighing of the highly hygroscopic dried material showed gains of from 1 to 2 mg. per minute depending on the prevailing humidity.

If optical activity is to be taken as a criterion of the purity of beta amylose solutions, the following table serves to show that the solutions used in these experiments are of a purity which compares favorably with the materials used in other researches.

TABLE IV

The Optical Activity of Beta Amylose Solutions.

Sample	Rotation (2 dm)	Concentration mg. per cc.	5461.Å	A_D
F-1	20.97 degs.	47.2	221.7	187
F-3	20.68 "	45.7	226.2	192
F-9	14.28 "	31.8	224.0	189
F-11	10.67 "	24.0	222.5	188
E-B4P	14.04 "	35.5	219.0	186
E-B4	16.80 "	37.3	225.0	191
B-1	12.68 "	29.1	218.0	185
E-B5	24.35 "	53.8	225.0	192
B-3	12.70 "	29.1	218.0	185
				Av. 188

The solutions were so clear that the average deviation in the reading on the polariscope was 0.02.

The A_D was determined by conversion of the rotatory power calculated from observations made with the mercury vapor lamp by the use of the factor of Vosburg for fructose solutions.

These figures are in reasonable agreement with those of Fouard[5], from 181° to 190°; of Taylor and Iddles[1], from 182° to 188°; and of Samec[6], 189°, for beta amylose solutions.

The preliminary experiments on re-solution of the corn beta amylose had indicated that there was a certain amount of material which would not dissolve. In order to test the effect of prolonged heating, experiments were run under constant volume conditions. A flask containing a suspension of crude thiocyanate-free corn beta amylose which had not been freed of gamma amylose was fitted with a reflux condenser and stirrer with a seal to prevent evaporation. A siphon was arranged by which samples could be withdrawn at any time without change of concentration due to opening the system. The mixture being constantly stirred was uniform. The flask was heated and samples withdrawn as soon as boiling took place and at the indicated intervals after boiling started.

For convenience, in run I, 15 grams of beta amylose were added to 325 cc. water, and in run II, 20 grams were added to 500 cc. water. A moisture determination had shown the carbohydrate to contain 18% water. So that the maximum concentration of solution possible if the entire sample of dry carbohydrate were beta amylose was for sample I 37.2 mg. per cc. and for sample II 32.8 mg. per cc. The time is expressed in minutes after the solution started to boil—the first sample being taken at that point.

In each case after cooling the precipitation of some amylose took place and the dry weight determination of the clear supernatant liquid was made the following day. Qualitative tests on the supernatant liquid showed increasing amounts of reduction with Fehling's solution with length of time indicating some decomposition of the beta amylose on excessive boiling. After precipitation had taken place there was no further clouding up of the solution, thus indicating no change in the beta amylose on standing. Quantitative tests on this point will be given later.

TABLE V

The Solubility of Crude Beta Amylose on Prolonged Heating.

RUN I			*RUN II*		
Sample	Time	Conc. mg. per cc.	Sample	Time	Conc. mg. per cc.
A	0	33.3	F	0	28.8
B	35	34.5	G	61	29.3
C	109	34.8	H	186	29.5
D	204	35.0	J	238	30.1
E	403	35.8	K	360	30.1
			L	685	30.4

The steady increase in the concentration of the solution might mean slow solution of the amylose. While this is possible it is felt that a loss of solvent due to slight evaporation over the long period of heating affords a more reasonable explanation than an increase in the amount of solute.

This is supported by the observation that where the duration of heating is comparable the increase in concentration is the same. The difference in length of time of heating between samples B and E is 368 minutes, while the difference in concentration is 1.3 mg. per cc. The difference in time of heating between samples F and K is 360 minutes, while their difference in concentration is also 1.3 mg. per cc. Were the increase due to the solution of more of the amylose, it would be proportional to the amount of carbohydrate taken originally. It would seem that approximately 90% of the crude corn beta amylose was soluble and that the other 10% was an insoluble amylose. The appearance of the solutions, A, E, F, and L, was the same.

If the crude beta amylose contains gamma amylose which does not go into solution because the gelatinizing ammonium thiocyanate has been removed, the beta amylose when pure should be completely soluble. Excessive drying should not affect its solubility, although this has been considered a possible explanation of the incomplete solution just discussed.

To test this question a solution of corn beta amylose was made up in the usual way from the crude product and after the customary settling had taken place the clear solution of beta amylose was siphoned off and reprecipitated with alcohol. The white taffy-like product was treated with six successive washings of 95% alcohol—each standing about 12 hours—without stirring with a rod, but with frequent agitation. If this product had been heated in order to dry it, a hard horny mass, difficult to powder in a mortar, would have resulted, whereas by this method of gradual dehydration a fine, white powder is obtained. The fine white, powdery, pure beta amylose was then treated with three extractions of absolute alcohol and washed free of the alcohol with two treatments of ether. Finally the very pure and very dry beta amylose was kept for 15 hours at 85° C. in the oven. This solid dissolved completely in water and no residue appeared as in the case of the unpurified (or un-reprecipitated) beta amylose.

The concentration of this clear solution was about 35.0 mg. per cc. which is sufficient to show that any precipitation from crude beta amylose solutions of this concentration is not due

to supersaturation at room temperature. This highest concentration of corn beta amylose solution actually determined by this worker was 69.3 mg. per cc.

The fact that this reprecipitated corn beta amylose is completely soluble at ordinary temperatures, although very dry, shows that its solubility is not a function of its moisture content and at the same time points toward the presence of the insoluble gamma form in the crude beta amylose. This gamma amylose is apparently precipitated along with the beta amylose after the ammonium thiocyanate treatment and electro-phoresis of the alpha amylose. The presence of the gamma amylose, therefore, seems to be due to the part played by the ammonium thiocyanate used in the process of gelatinization. Evidently the presence of ammonium thiocyanate causes the solution of gamma amylose with the beta amylose and its removal allows the gamma amylose to precipitate. It was thought desirable to study the effect of other agents, as well, in causing the solution of the gamma amylose along with the beta amylose. In the following experiment various salts were used for the purpose.

A suspension of 30 grams of crude beta amylose in 750 cc. of water was heated to 93° C. and before any precipitation took place 50 cc. samples were placed in "Non-Sol" bottles after which various salts were added separately as follows: the solutions were made normal to sodium chloride, to potassium chloride, to ammonium thiocyanate, to sodium thiosulfate, to aluminum sulfate, to disodium hydrogen phosphate, to sodium carbonate, to potassium iodide and also half normal to potassium iodide respectively. On standing the usual precipitation occurred, so far as could be observed to the same extent except in the cases of ammonium thiocyanate and potassium iodide.

This experiment tends to confirm the theory explaining the presence of the insoluble corn gamma amylose in the soluble corn beta amylose. It has already been stated that a large excess of ammonium thiocyanate was present during the separation by electro-phoresis of the alpha and beta amyloses. Since it has been shown in the above experiment that the gamma amylose does not precipitate in the presence of gelatinizing salts[7], it is supposed that this gamma form is carried along with the beta amylose in solution. When the carbohydrate was precipitated with alcohol, care was taken to free it as completely as possible from ammonium thiocyanate. The amount of ammonium thiocyanate as shown by the very faint color

given by ferric chloride solution was estimated to be one part in two thousand. The gamma being thus freed from the material which held it in solution, probably as a complex, does not dissolve permanently when the solution is made up and precipitates out on cooling. Attention is called at this point to the fact that reprecipitated corn beta amylose was shown to be completely soluble.

In an effort further to substantiate this theory of the effect of the gelatinizing salts, experiments were conducted as follows:

To 150 cc. of water was added 14.55 g. of potassium thiocyanate and then with stirring and heating 6.3 g. crude unpurified beta amylose. The solution was about normal to potassium thiocyanate and contained about 30.0 mg. of amylose per cc. A clear solution resulted—that is, there was no precipitate, as there would have been without the addition of the potassium thiocyanate. This same concentration of crude beta amylose was cloudy in the absence of potassium thiocyanate. This was divided into two approximately equal parts—one being kept as the reference sample. To the other was added 12.8 g. of silver nitrate (enough to ensure the complete precipitation of the potassium thiocyanate). A very heavy precipitate of silver thiocyanate and amylose was thrown down together. The solution was filtered and remained unchanged for over eight months. It showed the presence of silver ion but not of thiocyanate ion. Subsequently the clear unaltered solution which had been kept as a control was put in a U-tube and electrophoresed. A large precipitation of amylose resulted. This seems to show that the gamma form is held in suspension by the gelatinizing medium.

The question of salting out and "dragging down" was investigated. Clear, ultrafiltered solutions of crude corn beta amylose made up with varying concentrations of ammonium thiocyanate as high as 2M were treated with calculated equivalent amounts of copper sulfate. In each case the black copper thiocyanate was precipitated and at the same time a white cloud was dispersed throughout the solution and slowly settled. This seemed to show what had occurred on the electrophoresis—that on destruction of the gelatinizing ion the insoluble compound was free to precipitate. But when a blank solution, made up with no thiocyanate, was treated with two normal copper sulfate (there was no salting out at this point) and then ammonium thiocyanate added in equivalent amount, the same phe-

nomenon was observed. For this reason the experiments on the destruction of the gelatinizing agent by its precipitation cannot be held rigorously.

The same results were obtained using potassium iodide as gelatinizing agent. On destruction of the potassium iodide by oxidation, etc., precipitation took place. It was noticed that solutions of pure beta amylose treated with iodine test solution finally settled leaving a clear supernatant liquid. Raw corn starch when treated with potassium iodide or ammonium thiocyanate remained in "solution" or complex formation for over three months without any sign of precipitation.

The so-called gamma amylose was isolated in this way. The supernatant liquid above the electrodialyzed alpha amylose—in the separation by the method of Taylor and Iddles (loc. cit.)—and having a high concentration of ammonium thiocyanate, was siphoned off and precipitated with alcohol. The precipitate was washed with alcohol until the supernatant liquid gave no test for thiocyanate ion with ferric chloride solution. It was then dried by washing with 95% alcohol or acetone, filtered and finally washed with ether. This precipitated crude beta amylose was then taken up in water with stirring and heating to give an opalescent solution. (The concentration ranged from 24 to 69 mg. per cc.) The solution thus formed was practically clear at high temperatures (about 90° C.). On cooling there was no immediate precipitation. After about an hour however flocculation took place and in the course of a day or so the entire precipitate had settled. The supernatant solution (presumably of true beta amylose) showed all the properties described by Taylor and Iddles (loc. cit.). After the precipitate (which is considered the gamma amylose) had settled the solution above was siphoned off and distilled water added. Upon the settling of the gamma amylose the operation was repeated until the washings (when acidified) showed no blue color with iodine test solution. This highly flocculent precipitate was then treated with several portions of alcohol and finally washed with absolute alcohol and ether, filtered and dried. The resulting solid is a white powder differing but little, if at all, from beta amylose in appearance.

A more refined experiment on the precipitation of amylose with the destruction of the gelatinizing agent was carried out. A suspension of 16 grams crude beta amylose was made in 500 cc. of water and ammonium thiocyanate added until the solution was normal to it. On heating a solution was obtained

which was filtered. The clear filtrate was put in a 12-inch U-tube and the solution electrolyzed using collodion covered electrode chambers in which the water was changed frequently. The passage of current was slow due to the high resistance of the membrane, but in time with the decrease of the concentration of the ammonium thiocyanate precipitation of gamma amylose took place.

A clear solution of pure corn beta amylose was electrodialyzed to determine whether this precipitation was due to oxidation of the soluble amylose as has been suggested by Gruzewska[8], but after three weeks with no change in the solution the experiment was discontinued on the assumption that no reaction would take place.

Since the above solution containing the ammonium thiocyanate was ultra filtered, there is no possibility that any insoluble material could be in the filtrate unless it is held in solution chemically as a soluble complex. It is therefore held that the corn gamma amylose is kept in solution by the gelatinizing agent and is in reality a third form of amylose differing in its properties from the other two as indicated above.

In suspension gamma amylose is extremely bulky, but when isolated and thoroughly dried it is a fine white amorphous powder—50 cc.s of highly flocculent material yield about 200 mg. of dry solid.

To a sample of gamma amylose from which all beta amylose had been removed iodine test solution was added. No blue color was observed. Sodium carbonate was added and the mixture boiled for about a minute—or until the solution was clear. The carbonate was exactly neutralized to litmus with N HCl and iodine test solution again added. This time the characteristic blue color of beta amylose appeared. These reactions are comparable with those reported by Ling and Nanji for amylohemi-cellulose[9].

EXPERIMENTS ON REACTIONS OF PURE CORN BETA AMYLOSE

Having established the fact that when crude beta amylose was taken up into solution the initial precipitation was due to incomplete separation of the soluble and insoluble forms because of the latter being held in solution by the gelatinizing salt, investigation was made into the possibility of reactions taking place with pure corn beta amylose. This was done with the view of shedding further light on the corn starch problem and in the hope that it might suggest a means of attacking the

question of the retrogradation of whole starch pastes.

The factors reported to facilitate the formation of retrograded starch are:

(1) Concentration (Bakhuyzen[10], Maquenne[11])
(2) Time (Roux[12], Fouard[13])
(3) Low temperatures (Bakhuyzen[10], Gruzewska[14], Maquenne & Roux[15], Sherman & Baker[4])
(4) Alkalinity (Maquenne[16])
(5) Strong acids (Fouard[17])

Therefore the effect of each of these factors on pure corn beta amylose solutions was studied separately and in addition the actions of neutral, acid and basic salts, of salts which have high gelatinizing capacity, and the behavior of the amylose solution when subjected to direct current potential.

The beta amylose used in these experiments was made in the usual manner from ammonium thiocyanate-treated corn starch. The concentration of solutions is expressed in milligrams of carbohydrate per cubic centimeter of solution.

In these experiments control samples were always kept and their records constitute the data for the following experiment. In studying the effect of concentration and ageing the solutions were kept at room temperature in "Non-Sol" bottles and covered with a thin layer of toluene to prevent mould growth.

The results indicate the stability of beta amylose solutions.

TABLE VI

Showing the Effect of Ageing on Beta Amylose Solutions.

Sample	Start	Conc. mg. per cc.	End	Conc. mg. per cc.	Elapsed Time	Difference in mg. per cc.
1	Nov. 6	47.0	May 14	47.1	189 days	0.1
2	Dec. 1	34.5	May 12	35.0	163 "	0.5
3	Dec. 3	29.8	May 13	29.3	161 "	0.5
4	Dec. 3	33.4	May 25	31.8	177 "	1.6
5	Dec. 16	24.0	May 25	23.8	160 "	0.2
6	Mar. 11	29.1	Dec. 17	29.0	281 "	0.1
7	May 14	31.9	May 25	31.8	11 "	0.1
8	May 23	29.6	July 2	29.6	39 "	0.0
9	May 23	29.6	Dec. 3	28.1	194 "	1.5
					Av. change	0.5 mg.

These clear solutions remaining unchanged at widely different concentrations from 24.0 mg. per cc. to 47.0 mg. per cc. and over such long periods of time indicate that corn beta amylose does not revert. These results are in accord with the prediction of Maquenne[16].

Several investigators (see above) have described the effect of low temperature on starch pastes. In considering this factor several sets of experiments were run and are described below.

A solution of pure corn beta amylose was made from the crude beta amylose, ultrafiltered concentration determined to be 33.2 mg. per cc. and treated as follows: To 50 cc. samples of this solution were added from 2 to 15 cc. of tenth normal hydrochloric acid in varying amounts and from 2 to 8 cc. tenth normal sodium hydroxide. A solution containing potassium iodide was normal with respect to potassium iodide. The solutions were kept at room temperature for 126 days and then placed in the ice box at about 7° C. for 59 days. There was no visible change in any of the solutions in this entire time except the one containing potassium iodide which (due to oxidation of potassium iodide) gradually turned brown and then blue.

The indications were that temperatures as low as 7° C. do not effect the formation of an insoluble material from pure corn beta amylose. A quantitative test was run to determine the difference if any between clear ultrafiltered solutions of corn beta amylose kept at room temperature and in the ice box with low concentration of hydrochloric acid. A solution whose concentration was determined to be 32.3 mg. per cc. was divided into 2 sets of 3 samples of 50 cc. each. One set was kept at room temperature, the other in the ice box at about 7° C. for 45 days. Dilute hydrochloric acid was added in calculated amounts until the concentration of hydrogen chloride in two samples was five hundredth normal and in two samples two hundred fiftieth normal. In determining the dry weight the acid was neutralized with standard solution of dilute sodium hydroxide and the salt formed allowed for. The results follow:

TABLE VII

Showing the Effect of Dilute Acid on Pure Corn Beta Amylose Solutions at Two Temperatures.

Treatment	Final Conc. at Room Tem. mg. per cc.	mg. Difference from Original	Final Conc. at 7° C. mg. per cc.	mg. Difference from Original
Blank	31.8	— 0.5	31.4	— 0.9
N/500 HCl (final conc)	32.8	+ 0.5	31.8	— 0.5
N/250 HCl (final conc)	32.7	+ 0.4	32.7	+ 0.4

Temperatures as low as 7° C. have no effect on the formation of insoluble material from pure corn beta amylose solutions when mildly acid.

A quantitative study of the effect of dilute hydrochloric acid and sodium hydroxide comparable with the results obtained above was made using a solution of similar preparation and concentration (found to be 33.4 mg. per cc.) but not ultrafiltered. Samples were taken while the solution of crude corn beta amylose was hot and homogeneous and treated with reagents in exactly the same manner, viz: from 2 to 15 cc. of tenth normal hydrochloric acid or from 2 to 8 cc. of tenth normal sodium hydroxide were added. One sample was made normal to potassium iodide. Precipitation of the gamma amylose took place in the usual way. The acid treated samples precipitated first (Sherman & Baker, loc. cit. p. 1890) and shortly afterward those containing alkali. The amount of precipitate (by difference) was the same. There was no precipitation in the sample treated with potassium iodide. The solutions stood at room temperature for 159 days after which their concentrations were determined again. In these cases it was necessary to make corrections for (1) the salt formed after neutralization was effected to prevent charring of the carbohydrate during the dry weight determination and (2) change of volume (and hence of concentration) of the solution brought about by the addition of the acid or alkali. Since in sample "G", 8 cc. of alkali solution was added to 50 cc. of beta amylose solution the concentration of the latter is now $^{50}/_{58}$ of the original concentration. The dry weight was made as usual with 10 cc. of solution so that the actual dry weight obtained after correction for the sodium chloride formed in neutralization is multiplied by $^{58}/_{50}$ to give a result comparable with the original concentration which was 33.4 mg. per cc. This explains the term "corr. conc." in the following table:

TABLE VIII

Showing the Effect of Dilute Acid and Alkali on Solutions of Crude Corn Beta Amylose at Room Temperature.

Sample 50 cc.	Treatment	Actual Conc. mg. per cc.	Corr. Conc. mg. per cc.	Difference mg. per cc.
A	Blank	31.9	31.9	1.5
B	2 cc. N/10 HCl	31.7	33.0	0.4
C	15 cc. N/10 HCl	25.5	33.2	0.2
D	2 cc. N/10 NaOH	31.9	32.9	0.5
E	4 cc. N/10 NaOH	30.7	32.7	0.7
F	6 cc. N/10 NaOH	30.1	33.1	0.3
G	8 cc. N/10 NaOH	29.5	33.4	0.0
			Average	0.5

These results indicate no change in the pure (supernatant) corn beta amylose solutions upon treatment over a long period with dilute alkali or acid.

The effect of non-gelatinizing salts with a solution of pure corn beta amylose, whose concentration was 24.0 m. per cc., and which had been ultrafiltered is shown by the following experiments. Salts were added to 50 cc. samples until their concentration was that indicated in the column headed "treatment". The results which follow could not be measured satisfactorily because of the decomposition of the carbohydrate. Although qualitative in nature they are in line with the experiments described above.

TABLE IX

Showing the Effect of Non-Gelatinizing Salts on Pure Beta Amylose Solutions.

Sample	Treatment	RESULTS AFTER STANDING					
		2 Days	4 Days	14 Days	19 Days	1 Mo.	2 Mos.
a	Blank	Clear	Clear	Clear	Clear	Clear	Clear
b	M to $Al_2(SO_4)_3$	Clear	Cloudy	Slt. ppt.	Slt. ppt.	Slt. ppt.	Slt. ppt.
c	M to Na_2HPO_4	Clear	Clear	Clear	Clear	Clear	Clear
d	M to KCl	Clear	Clear	Clear	Clear	Clear	Clear
e	M to NaCl	Clear	Clear	Clear	Clear	Clear	Clear
f	M to $AgNO_3$	Clear	Clear	Clear	Clear	Clear	Clear
g	M to $NaHCO_3$	Clear	Cloudy	Cloudy	Cloudy	Slt. ppt.	Slt. ppt.
h	$\frac{1}{4}$M to Na_2CO_3	Clear	Clear	Cloudy	Cloudy	Slt. ppt.	Slt. ppt.
j	$\frac{1}{2}$M to Na_2CO_3	Clear	Clear	Cloudy	Cloudy	Slt. ppt.	Slt. ppt.
k	M to Na_2CO_3	Clear	Cloudy	Cloudy yellow	Cloudy yellow	Ppt. yellow	Ppt. yellow
l	2M to Na_2CO_3	Clear	Crystals yellow	Cloudy yellow	Cloudy yellow	Dark	Red
m	3M to Na_2CO_3	Cloudy	Crystals yellow	Cloudy yellow	Dark	Red	Red

In sample "m" there was evidence of salting out as well as crystal formation.

When acids were added to the crude unpurified beta amylose they coagulated the insoluble portion so that it precipitated much more quickly than without the acid[17]. On the other hand, the gelatinizing salts caused no precipitating at all when in sufficient concentration; i. e., one mole to about 4 gms. carbohydrate.

Alkali when very dilute (about five hundredth normal) also had a precipitating effect, but soon this changed to dissolving power as the concentration was increased. Although several workers—Ling and Nanji[18], Maquenne (loc. cit.) and Samec[19] —have claimed that this was compound formation between the starch itself (or in this case the pure beta amylose) and the alkali, it was found that on prolonged standing (more than a year) a heavy precipitate was formed which seemed to be sodium oxalate. This is true only of solutions of beta amylose as strong as three molar to sodium hydroxide and although in a two molar solution there was decomposition, as evidenced by change of color to a strong reddish tinge, there was no precipitation. Armstrong[50] cites the possible decomposition of starch by alkali into more than one hundred different products.

The following experiment was performed to explain why certain workers have considered that cooling increases the speed and degree of retrogradation of starch pastes.

A solution of crude beta amylose was made up in the usual manner by heating until at about 87° C. the suspension became opalescent. Then instead of allowing the solution to cool slowly at room temperature, it was immediately placed in the ice box at about 7° C. In place of the customary precipitate settling out at the bottom, the entire liquid turned into a thick white paste which did not settle or alter in more than six months. Two samples of this paste were heated to opalescence again and treated differently. One was cooled slowly to room temperature and allowed to stand while the other was replaced in the ice box immediately. The former solution slowly clouded up and precipitated exactly as had always taken place in these unfiltered samples of crude beta amylose, while that which was placed in the ice box returned as nearly as could be determined to the thick pasty condition from which it had come.

A large proportion of this paste was readily soluble in dilute hydrochloric acid, was colored blue with iodine test solution and showed the other properties ascribed to beta amylose.

This, with the experiments on "dragging down" of beta amylose when the thiocyanate ion was precipitated with copper sulfate solution (since the precipitation with cooling was rather rapid), would indicate that when the alpha or gamma amylose is precipitated, a large portion of the beta amylose is occluded and otherwise held with the insoluble form in its precipitation.

It seems that irrespective of the amount of precipitate due to the difference of method of precipitation, the total soluble constituent when the whole mass was heated up and allowed to cool slowly, was sensibly the same.

Some additional observations which were made in the course of the work follow:

(1) Pure corn beta amylose solutions were unaffected by buffer solutions between pH values from 5.2 to 9.6.

(2) Corn beta amylose solutions dialyzed with apparent ease through a collodion sac which had been hardened by drying in the air for three days.

(3) The gamma form when dissolved in sodium hydroxide and exactly neutralized with hydrochloric acid does not re-precipitate, which seems to disagree with the theory that a stable compound is formed between the amylose and the alkali (see above).

(4) As the beta amylose concentration is decreased by decantation from a crude beta amylose solution the gamma amylose settles more rapidly.

SUMMARY

1. There is apparently a corn gamma amylose which comes from corn alpha amylose.

2. The corn gamma amylose is held in clear solution with corn beta amylose by ammonium thiocyanate.

3. Corn beta amylose does not revert to an insoluble form.

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VITA

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